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Estradiol-induced potentiation of dopamine release in dorsal striatum following amphetamine administration requires estradiol receptors and mGlu5

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Title page

Estradiol-induced potentiation of dopamine release in dorsal striatum following amphetamine administration requires estradiol receptors and mGlu5

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Abstract

Estradiol potentiates behavioral sensitization to cocaine as well as self-administration of cocaine and other drugs of abuse in female rodents. Furthermore, stimulated dopamine (DA) in the dorsolateral striatum (DLS) is rapidly enhanced by estradiol, and it is hypothesized that this enhanced DA release mediates the more rapid escalation of drug taking seen in females, compared with males. The mechanisms mediating the effect of estradiol to enhance stimulated DA release was investigated in this study. Using in vivo microdialysis and high performance liquid chromatography coupled with electrochemical detection, we first examined the effect of estradiol on amphetamine-induced DA increase in the DLS of ovariectomized rats. We then tested if the potentiation of this DA increase could be blocked by the estradiol receptor antagonist, ICI 182,780 (ICI), or an antagonist to the metabotropic glutamate receptor subtype 5 (mGlu5), 2-Methyl-6-(phenylethynyl)pyridine (MPEP). There is evidence that estradiol receptors collaborate with mGlu5 within caveoli in DLS and mGlu5 is hypothesized to mediate many of the effects of estradiol in the addiction processes in females. Our data show that estradiol enhances the DA response to amphetamine. Either ICI or MPEP prevented the effect of estradiol to enhance DA release. Importantly, our results also showed neither ICI or MPEP alone is able to influence the DA response to amphetamine when estradiol is not administrated, suggesting that ICI and MPEP act via estradiol receptors. Taken together, our findings demonstrate that estradiol potentiates amphetamine-stimulated DA release in the DLS and this effect requires both estradiol receptors and mGlu5.

Significance Statement

The present study provides important information on the neurobiological mechanisms underlying the exacerbating effects of E2 on addictive behavior by showing blockage of E2 receptors or mGlu5 reduces E2-induced potentiation of DA release in the rat striatum following by amphetamine injections. Our data suggest targeting E2 receptors or mGluRs could have treatment potentials for E2-related disorders in areas such as, but not limited to, drug addiction.

Introduction

Women are more susceptible to drugs of abuse than men. They escalate faster from initial use to addiction, take more drugs when addicted, and have a harder time staying abstinent (Bobzean, DeNobrega et al. 2014). This is mirrored in animal models, female rats acquire drug self-administration at a faster rate, are more motivated to take drugs, and respond stronger to drug cues during reinstatement (Becker 2016, Song, Kalyani et al. 2018).

It is suggested that these sex differences are regulated at least in part by estradiol (E2). Indeed, there is considerable evidence that shows the potentiating roles of E2 in cocaine self-administration, cocaine behavioral sensitization, and dopamine (DA) signaling in the nucleus accumbens (NAc) following cocaine administration (Hu and Becker 2008). Despite this mounting evidence, how E2 enhances stimulated DA release or addiction-related behaviors are less well understood.

Many of the E2 effects involve intracellular estrogen receptors ERαs and ERβs (Foster 2012, Borrow and Handa 2017). Recently, E2 is also shown to bind to a membrane G protein coupled receptor GPER-1 (Long, Serey et al. 2014). Depending on the types/locations of the receptors, the effects of E2 can range from minutes (non-genomic effects) to days (genomic effects) (Ervin, Lymer et al. 2015). In dorsal striatum (DS), a region that is critical for habitual drug taking behavior, E2 modulates behavior by acting on GABA medium spiny neurons (MSNs) (Mermelstein, Becker et al. 1996) and by altering DA transmission indirectly through a presynaptic mechanism (Xiao and Becker 1998, Schultz, von Esenwein et al. 2009).

In the present study by using *in vivo* microdialysis and high-performance liquid chromatography (HPLC) coupled with electrochemical detection (ECD), we first examined effects of E2 on amphetamine (AMPH) induced DA elevation in the striatum of female rats. We then tested if the observed potentiated DA elevation could be blocked by an E2 receptor antagonist ICI 182,780 (ICI) or an antagonist to the metabotropic glutamate receptor subtype 5 (mGlu5), 2-Methyl-6-(phenylethynyl)pyridine (MPEP) in the

striatum as there is evidence that mGlu5 is required for many of the effects of E2 in addiction processes (Martinez, Peterson et al. 2014, Martinez, Gross et al. 2016).

Materials and Methods

Animals. Female Sprague-Dawley (SD) rats (weighting 200-225g at the beginning of each experiment; were obtained from Harlan, Indianapolis, IN, or Charles River, Cambridge, MA) and housed in groups of 2 or 3 per cage before cannula implantation and singly housed after cannula implantation, under a 14:10 light/dark cycle. The rats were housed in a room maintained at a constant temperature of 20-21^{o}C, with phytoestrogen-free rodent chow (2014 Teklad Global, 14% protein rodent maintenance diet, Harlan rat chow; Harlan Teklad, Madison, WI) and water available *ad libitum*. All procedures were performed according to the protocol approved by the Committee for Use and Care of Animals at the University and were in accordance with the NIH Guide for Care and Use of Laboratory Animals.

Ovariectomy (OVX). About 1 week after arrival, all animals underwent bilateral OVX. The OVXs were conducted using a dorsal approach under anesthesia of about 2% isoflurane/oxygen. The skin was opened with an incision about 1cm long along the midline just below the ribs, and a small incision about 0.5cm long was made through the muscle 1.5-2cm lateral to the midline. The ovary was externalized with blunt forceps, and the tissue between the ovary and uterus was clamped with a hemostat. The ovary was removed, and the hemostat remained in place until there was no bleeding before being released. The uterus with associated tissue was then returned to the abdomen. The procedure was repeated on the other side, and the wound was closed with 9 mm wound clips. The wound clips were removed after 14 days of ovariectomy. After 7 days of recovery, all animals underwent vaginal lavage testing daily for 10 consecutive days to confirm cessation of cycling.

Cannula implantation. Two to three weeks after OVX, all rats received buprenorphine (0.01 mg/kg s.c.) or carprofen (5 mg/kg, s.c) 30-60 min ahead of the cannula implantation surgery. During the surgery, all rats were anesthetized with ketamine (60 mg/kg, i.p.) and dexmedetomidine (0.3 mg/kg, i.p.). Guide cannulae (matching for CMA/11 probes, from CMA/Microdialysis, Solna, Sweden, or MAB 6 probes, from SciPro, NY, USA; 4mm membrane length) were inserted through the skull aimed at the striatum (AP +0.20 mm, ML ±3.00 mm, DV -1.50 mm) using standard stereotaxic techniques. The cannulae were held in place with acrylic polymer (Lang, Wheeling, IL.) which was secured to the brain with 3-4 stainless steel jewelry screws (Small Parts, Miami Lakes, FL.). A solid stylet was placed in each cannula when not in use, in order to keep the cannula patent. Animals were allowed to recover for at least 5 days prior to microdialysis. Starting one day after the surgery (both cannula implantation and OVX), rats were administered with carprofen (5 mg/kg, s.c) daily for 3 consecutive days and triple antibiotic was given when necessary upon observation. All rats were observed at least once daily for 10 consecutive days to ensure their recovery.

Preparation for Microdialysis. Animals were anesthetized with 3% Isoflurane and maintained with 2% isoflurane during the procedure of removing the stylet and inserting a microdiaysis probe into the brain through the guide cannula. Probes were placed into the brain 12-18 hrs in advance of the testing to allow sufficient time for the injury-related release associated with probe implantation to subside. Animals were placed in the test chamber (31.0 cm x 25.0 cm x 25.0 cm) with continuous white noise. The microdialysis probes were attached to syringes mounted on the syringe pump, and a Ringer's solution (145 mM NaCl, 2.7 mM KCl, 1 mM $MgSO_4$, 1.2 mM $CaCl_2$, 1.55 mM Na_2HPO_4, 0.445 mM NaH_2PO_4, pH 7.3 at RT) was continuously pumped through the probe at 1.5 µl/min during the first 30-60 min after probe insertion. Then the pumping speed was reduced to 0.3 µl/min until the next day. To prevent the microdialysis probe, which was secured to the animals' head, from being subjected to the torque created during the movement of animal, the rats was fitted with a custom-made harness, and the harness was attached to a swivel (liquid commutator 375/22 or 375/D/22 from Instech Laboratories Inc., Plymouth

126 Meeting, PA) by a flexible stainless steel cable. Rats were left overnight in the testing chamber with food
127 and water freely available.

128 **Microdialysis.** Sample collection was initiated the next morning, and all samples were collected in
129 the light phase during 8:00 – 12:30. All dialysates were briefly stored on ice in dark, and then manually
130 injected into HPLC-ECD system for measuring DA concentration in dialysates during 8:00 – 15:00 of the
131 same day. Dialysate was collected into vials mounted just above the harness assembly. Drugs and
132 hormones of interest were administered systemically (i.p. or s.c.) or intrastriatally via the microdialysis
133 probe (reverse dialysis). For delivering E2, ICI, or MPEP via reverse dialysis method, drugs were first
134 dissolved in pure UPS grade ethanol as 1000x (or above) stock solution; then, at use, they were further
135 freshly diluted in Ringer's solution and manually filtered via 0.2 um syringe filters. With reverse dialysis,
136 the drug of interest passes through the membrane of a microdysis probe and diffuses into the striatum
137 down a concentration gradient. Based on the *in vitro* results, we estimate that the efficiency of drug
138 delivery with infusion method is 3-10% (data not shown). Thus, the effective concentration in the brain is
139 considerably lower than the concentration in the probe. Thirty - sixty minutes before the first sample
140 collection, the pumping speed was increased to 1.5 µl/min. Each dialysate sample was collected for 10
141 min. Baseline samples were collected for thirty minutes. When drugs were delivered via reverse dialysis,
142 five samples were collected after the solutions were changed and the last three samples were used as the
143 new baseline (it took about 20 min for a new solution to reach equilibrium in the system). All rats in all
144 experiments received an AMPH injection during microdialysis (2.5 mg/kg in saline, i.p.) and 10-min
145 samples were collected for the following 2 hours (12 samples).

146 Treatment protocols for each experiment prior to AMPH administration. See Fig.1 for treatment
147 details during the microdialysis sample collection in each experiment. Briefly, all rats were infused with
148 Ringer's solution for determining baseline DA and then treated with one or two pre-treatments prior to
149 AMPH injections. Specifically, in Experiment 1, rats were randomly assigned to 1 of 4 groups: (1) E2 Group

(n=7), rats were infused with Ringer's solution with E2 in it (1 ng/ml E2; first dissolved in 100% ethanol and then diluted in Ringer's solution, ethanol final concentration 0.02%); (2) Estradiol benzoate Group (n=8), rats were treated with a subcutaneous (s.c.) injection of EB (5 µg in 0.1 ml peanut oil); (3-4) Control Groups, rats received either a subcutaneous (s.c.) injection of peanut oil (0.1 ml per rat, n=6) or 0.02% ethanol in Ringer's solution (vehicle for E2, n=7). Rats that were treated with peanut oil or ethanol in Ringer's solution did not significantly differ from each other and were combined in the analyses. There were two groups in Experiment 2: the ICI Group (n=9) was infused with Ringer's solution with ICI in it (2.32 µg/ml ICI, which is an equimolar concentration to E2 1 ng/ml; first dissolved in 100% ethanol and then diluted in Ringer's solution; ethanol final concentration was 0.1%). The rats then received a s.c. EB injection following the ICI treatment. Control Group (n=8) received Ringer's solution with 0.1% ethanol (vehicle for ICI), followed by an EB administration. Experiment 3 also had two groups: E2 + MPEP Rats (n=9) received E2 via reverse dialysis as described above and an intraperitoneal (i.p.) MPEP injection (10 mg/kg). Control rats (n=9) received E2 via reverse dialysis and an i.p. saline injection. In Experiment 4, rats were assigned into 1 of the 4 groups: ICI group (n=7) where ICI dissolved in Ringer's solution was administered via reverse dialysis, MPEP group (n=6) where MPEP was injected systemically as above, and two control groups where rat received i.p. saline (n=4) or ICI vehicle (n=4). The two control groups were combined due to similar levels of baseline DA as well as DA concentrations following AMPH injections. All rats were injected with AMPH following these pre-treatments and dialysate samples from the DLS were collected every 10min for 2 consecutive hours.

DA concentration measurement by HPLC. DA concentration was assayed using a HPLC-ECD system described in (Hu and Becker 2003). In brief, dialysate samples were separated on an ESA (ESA biosciences, Chelmsford, MA) HPLC column (HR-80X3.2, 3 um particle size, 80mm length) at 40 °C, with a mobile phase consisting of: 75 mM NaH_2PO_4, 0.2 mM EDTA, 1.4 mM OSA (1-octanesul fonic acid sodium salt monohydrate, Fluka Cat#74882) and 17% methanol in HPLC water (PH4.7). Flow rate through

the column was set to 0.7ml/min. Dopamine was quantified using a coulometric detector (Coulochem II, ESA) equipped with a high sensitivity analytical cell containing dual coulometric working electrodes (ESA model #5014B). The detector settings were as follows: detector 1 -150 mV, detector 2 +100 mV, and guard cell +300 mV. Output from detector 2 was used for dopamine quantification. The retention time of DA was about 2.5 min.

Histology. Four-seven days following completion of microdialysis, animals received an overdose of anesthesia and were sacrificed. Their brains were prepared for histological analysis using standard techniques for frozen sections and Cresyl Violet staining was used to determine the location of the microdialysis probes. Only data from the rats where probes were located inside the DLS are reported here. Two rats were excluded due to the probes going too ventral and six more rats were also excluded due to probe damage or sickness.

Statistical analyses. We used software SPSS V24 in all data analyses. Data were expressed in mean±SEM. The percentage increase from baseline of each rat was used to assess DA response to AMPH in each 10-min sample. Baseline was determined by the mean of all samples before AMPH injections since no difference in DA concentrations was found in these samples (data not shown). Mixed Design Repeated-Measure ANOVA was used to examine treatment effect (e.g. ICI vs vehicle) among groups and the effect of time on DA concentrations within each group. We focused our analyses *a priori* on the first four samples collected following AMPH to catch patterns of peak DA concentrations in each condition. When significant effects of treatment were found, one-way ANOVA or t test was used to determine whether there was a significant difference in the each of the 4 samples post-AMPH among treatment group(s) and the control group. *A priori* planned contrast *post hoc* analysis was used to examine differences among more than 2 groups. Two data points in the first four samples post-AMPH of all rats (from 2 separate rats) were missing due to technical issues and were replaced by the average of the data

197 points right before and after. In cases when assumptions for parametric tests were not met,
198 nonparametric tests (e.g. Mann-Whitney U and Kruskal-Wallis tests) were used.

199

200

201 Results

202 Experiment 1. As can be seen in Figure 2, E2 delivered via reverse dialysis directly into the DLS or
203 EB s.c. significantly enhanced AMPH-induced striatal DA release relative to the control group. Repeated
204 Measures test showed there were a significant effect of treatment ($F(2,25)=4.659$, $p=0.019$) and a
205 significant interaction effect of Treatment x Time ($F(6,75)=3.640$, $p=0.003$) in the DLS DA concentrations
206 of the first 4 samples following AMPH injections. Planned *post hoc* comparison tests showed there were
207 significant effects of E2 and EB compared to controls ($p=0.009$, and $p=0.048$, respectively). To
208 understand better the time course on the differentiated elevation of peak DA levels among the three
209 groups, one-way ANOVA tests (and a priori planned post hoc comparisons) were used to compare each of
210 the 4 DA concentrations across conditions. Significant differences were found between the E2 and EB
211 treated rats and the control rats in the DA concentrations shortly after AMPH injections (See Table 1).
212 Experiment 2. As shown in Figure 3, ICI significantly decreased EB-induced enhancement in the
213 DA release in the DLS after an i.p. injection of AMPH. A significant main effect of Treatment was found in
214 the measured DA concentrations in the DLS ($F(1,15)=7.360$, $p=0.016$). There was also a significant
215 interaction between Treatment x Time (Repeated measures, $F(3,45)=4.045$, $p=0.010$). Mann-Whitney U
216 tests showed all the 4 samples collected after right after AMPH injections differed in DA concentrations
217 for ICI treated versus vehicle treated rats (assumptions for parametric t tests were not met, so non-
218 parametric tests were used, see Table 1).
219 Experiment 3. As shown in Figure 4, MPEP also significantly decreased EB-induced DA
220 potentiation in the DLS post AMPH treatment. There were a significant main effect of Treatment in the
221 DA concentrations (Repeated Measures, $F(1,16)=5.895$, $p=0.027$) as well as a significant interaction of
222 Treatment x Time in the DA concentrations (Repeated Measures, $F(3,48)=6.031$, $p=0.001$). Independent t
223 tests showed in nearly all samples after AMPH injections there were significant differences between rats
224 treated with MPEP versus those with saline in DA concentrations (see Table 1).

225 Experiment 4. As shown in Figure 5, MPEP or ICI did not influence DA release post AMPH when E2
226 was not administered in OVX rats, unlike what was seen in Experiments 2&3. The increase in DA
227 concentrations post AMPH administration did not differ in MPEP or ICI treated rats versus controls. There
228 was a significant effect of Time ($F(3,45)=16.300$, $p=2.598E-7$), but there was no main effect of Treatment
229 ($F(2,15)=0.140$, $p=0.870$) neither was there a significant interaction between Treatment and Time
230 ($F(6,45)=0.925$, $p=0.487$).
231 Lastly, as shown in Figure 6, there was no effect of EB, E2, ICI, or MPEP on the DA release in the
232 DLS prior to AMPH injections across all experiments. Paired t tests showed no difference between the
233 baseline DA concentrations and the DA concentrations after the administration of each of the above
234 agents prior to AMPH challenges ($t(4)=0.746$, $p=0.497$; $t(6)=0.544$, $p=0.606$; $t(7)=1.465$, $p=0.186$;
235 $t(4)=1.152$, $p=0.313$; for EB, E2, ICI, and MPEP, respectively).
236
237

Discussion

The present study showed E2 enhances DA release in the DLS following AMPH administration. This enhancing effect of E2 is mediated by E2 receptors and mGlu5 receptors as blocking E2 receptors in the DLS by ICI or i.p injections of mGlu5 receptor antagonist MPEPP inhibits the E2-induced DA elevation in DLS. We also showed ICI 182,780 and MPEP are not able to influence DA levels in the DLS when E2 is not administered in ovariectomized rats.

There is mounting evidence that E2 has been implicated in addictive behavior. E2 enhances ethanol reward in female mice (Hilderbrand and Lasek 2018). E2 is even found to increase choice of cocaine over food in male rats as observed in females (Bagley, Adams et al. 2017). Our data support the enhancing effect of E2 on reward and thus the notion that it exacerbates addictive behavior, as it increases dopamine levels in response to AMPH challenge. Interestingly, there is considerable evidence that estradiol reduces food intake in female rats (Yu, Geary et al. 2008, Butera, Wojcik et al. 2010, Santollo, Katzenellenbogen et al. 2010, Santollo and Daniels 2015, Butler, Hildebrandt et al. 2018) (but see (Boswell, Reid et al. 2006, Butera, Wojcik et al. 2010)). The mechanisms underlying the apparent differences in the roles of E2 in motivated behaviors are less well understood, but it could be that E2 acts in different brain regions to modulate different types of rewards (e.g. drug addiction versus food reward).

The ability of E2 in influencing addiction or reward may be due to its action in the midbrain dopamine reward system. Mice treated with E2 or ERβ agonists showed increased conditioned place preference for cocaine, while specific knockdown of the ERβ gene decreased cocaine conditioned place preference (Satta, Certa et al. 2018). Another study shows E2 acts on ventral tegmental area to increase the sensitivity of dopamine neurons to ethanol (Vandegrift, You et al. 2017). E2 in the MPOA also increases DA release in the NAc in response to cocaine (Tobiansky, Will et al. 2016). Our finding showed E2 in the DLS potentiates dopamine release following AMPH injections. DLS plays a critical role in addictive behavior in both rodent and human studies. In humans, damage to dorsal striatum alleviates

addiction to alcohol and nicotine (Muskens, Schellekens et al. 2012). In rodent studies, it has been suggested that dorsal medial striatum and NAc are crucial in the initial acquisition of the reward and then DLS and NAc begin to take over when the behavior becomes more addiction-like. Taken together, it is possible E2 acts on different regions to convergently modulate addictive behavior.

Both ERα and ERβ have been reported in the E2 modulation of addictive behavior. The ERα agonist (propyl-pyrazole triol (PPT)) and the ERβ agonist (diarylpropionitrile (DPN)), independently increased choice on the high-reward tested in an operant chamber (Uban, Rummel et al. 2012). These effects were most pronounced 24 h after administration suggesting genomic action of the receptors. Effects of E2 via its action on membrane receptors have been debated (Govind and Thampan 2003) and there is increasing evidence showing rapid effects of E2 that are likely via non-genomic receptors (Revankar, Cimino et al. 2005, Micevych, Wong et al. 2015, Paletta, Sheppard et al. 2018, Yoest, Quigley et al. 2018). E2 is found to exert its effects via acting on G protein coupled estrogen receptors (GPER-1) as well as ERα and ERβ receptors to rapidly facilitate short term memory in female mice (Lymer, Sheppard et al. 2018). Our finding in the present study showed E2 rapidly potentates dopamine release following AMPH treatment in the DLS. It will be important to further investigate the roles of each receptor type/location in these effects.

Several studies have showed that mGlu5 is involved in the effects of E2 in the regulation of behavior and physiology (Grove-Strawser, Boulware et al. 2010, Peterson, Mermelstein et al. 2015, Al-Sweidi, Morissette et al. 2016). E2 is reported to mediate dendritic spine plasticity in the NAc through activation of mGlu5 , evaluated via DiI labeling and confocal microscopy (Peterson, Mermelstein et al. 2015). The authors suggest E2's role in mediating neuronal plasticity in the NAc via mGlu5 is important for E2's effect in drug addiction. Another study shows E2 facilitates cocaine self-administration in ovariectomized rats and mGlu5 activation is essential for this effect (Martinez, Gross et al. 2016). The study also demonstrates direct activation of mGlu5 is insufficient to mimic the effect of E2 in cocaine self-

administration, suggesting E2 receptors possibly need to be activated simultaneously to have the effect. Taken together, these findings are consistent with the results of the present study that both E2 receptors and mGlu5 s are necessary for E2's potentiation in DA release in DLS. It will be important to extend these results by examining the involvement of mGlu5 in other E2-mediated behaviors.

While it is clear that both E2 receptors and mGlu5 are required for the estradiol evoked DA release from the DA terminals, our study does not show whether or not estradiol directly acts on or whether the two receptors are on the DA neurons. In fact, studies suggest estradiol activates E2 receptors coupled with mGlu5S on MSNs, which then modulates the release of GABA to influence DA terminals (Schultz, von Esenwein et al. 2009). E2 receptors can be anchored to plasma membrane via caveolin protein which then allow them to functionally couple with mGluRs (Yoest, Quigley et al. 2018). The authors propose that E2 and mGlu receptors collaboratively act on MSNs in the DLS to modulate DA release from DA neuronal terminals. It is also possible that E2 acts on other interneurons (such as cholinergic neurons) to modulate DA release in the DLS, or influences glutamate release on cortical afferents.

Our data demonstrated marked increase of DA release in DLS following AMPH injections. This effect has been reported both *in vivo* and *in vitro* in our previous studies (Becker and Ramirez 1981, Xiao and Becker 1998, Becker and Rudick 1999). Due to unknown vendor/batch effects, different magnitudes of overall increase in DA concentrations following AMPH administration were observed in Experiments 1&3 (rats from Harlan) than in Experiments 2&4 (rats from Charles River).

Conclusion

The present study demonstrate E2 directly potentiates the AMPH-induced increase in DA in the DLS. The effects of E2 are mediated by E2 receptors and can be blocked by an mGlu5 antagonist. These results provide important information on the neural mechanism through which E2 may contribute to sex

differences in behaviors such as, but not limited to, addictive behavior. Our data also suggest targeting mGlu receptors could be a potential treatment for E2 related disorders in female individuals.

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411 Table caption

412 Table 1: Comparisons of DA release in respond to AMPH among rats with varying pre-treatments in

413 Experiments 1,2&3.

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Figure caption

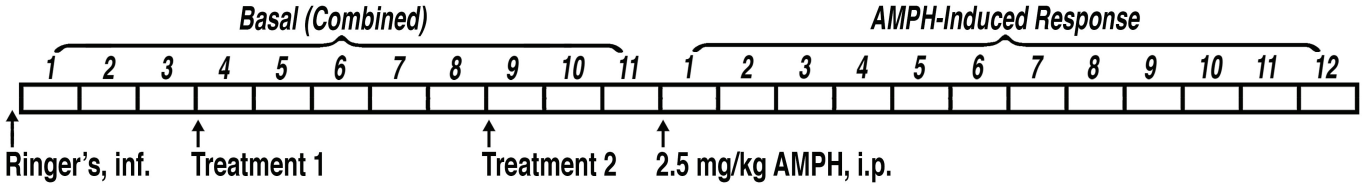
Figure 1 Schematic diagram of all the treatments in each experiment.

Figure 2 E2 in the DLS or EB s.c. potentiates DA release following AMPH injections. E2 was dissolved in Ringer's solution and was infused into the DLS via reverse microdialysis. EB: Estradiol benzoate. Note: the asterisk symbol * indicates a significant difference between rats treated with E2 or EB and control rats.

Figure 3 ICI infused into the DLS reduces E2-induced DA potentiation following AMPH injections. EB: Estradiol benzoate. Note: the asterisk symbol * indicates a significant difference between rats treated with ICI and control rats.

Figure 4 MPEP reduces E2-induced DA potentiation following AMPH injections. Note: the asterisk symbol * indicates a significant difference between rats treated with MPEP and control rats.

Figure 5 Neither ICI or MPEP influences DA release in the DLS when estradiol was not administered. These rats were ovariectomized and were not given EB injections or E2 infusions.



Exp 1	Treatment 1	Treatment 2	Group Size
Control I*	Not Apply	0.1 mL Peanut Oil, s.c.	n=6
Control II*	0.02% EtOH in Ringer's, inf.	1 mL/kg Saline, i.p.	n=7
EB	Not Apply	5 μg EB in 0.1 mL Peanut Oil, s.c.	n=7
E2	1 ng/mL E2 in 0.02% EtOH in Ringer's, inf.	1 mL/kg Saline, i.p.	n=8

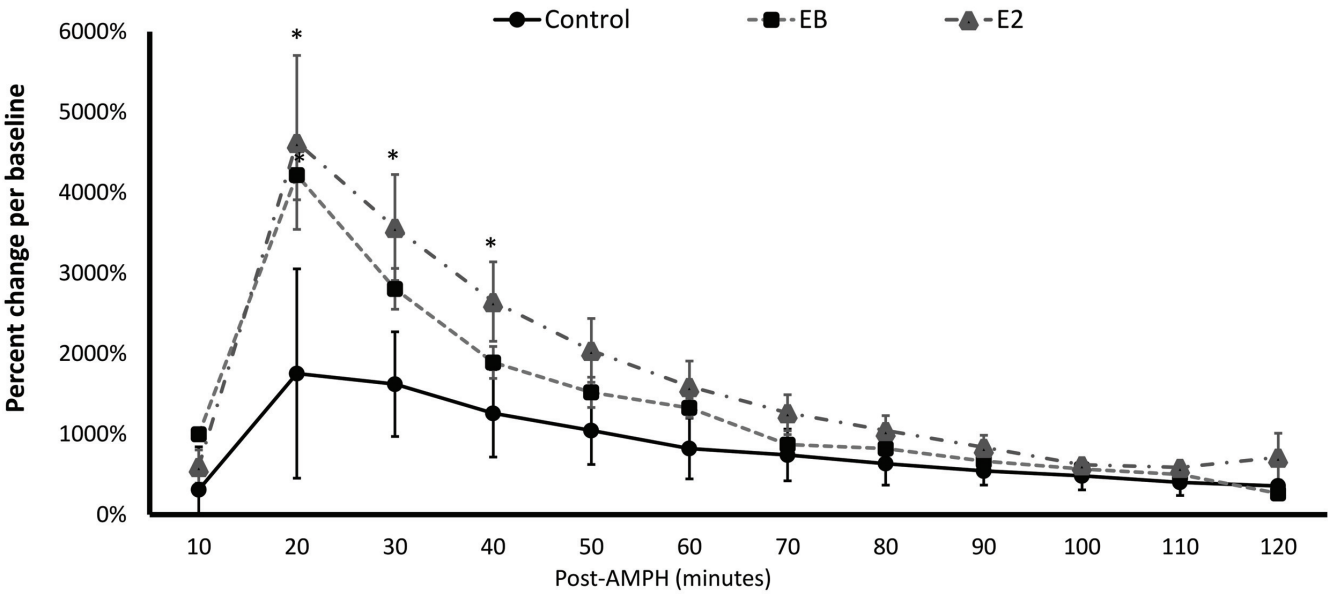
* Data combined

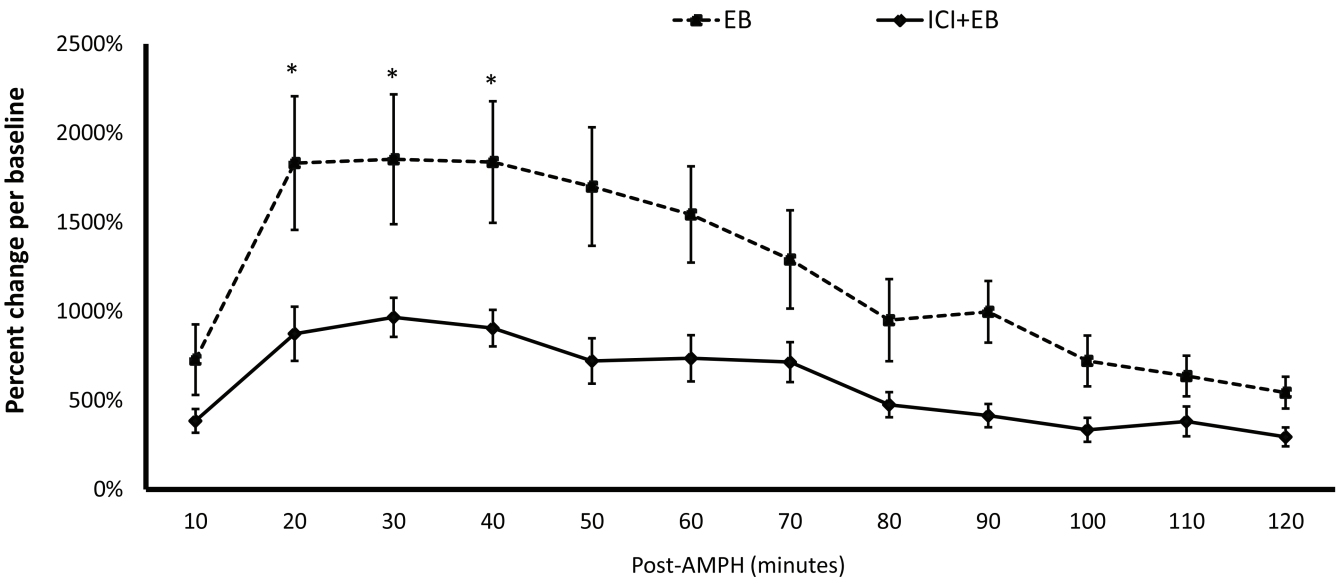
Exp 2	Treatment 1	Treatment 2	Group Size
EB	0.1% EtOH in Ringer's, inf.	5 μg EB in 0.1 mL Peanut Oil, s.c.	n=8
ICI+EB	2.32 μg/mL ICI in 0.1% EtOH in Ringer's, inf.	5 μg EB in 0.1 mL Peanut Oil, s.c.	n=9

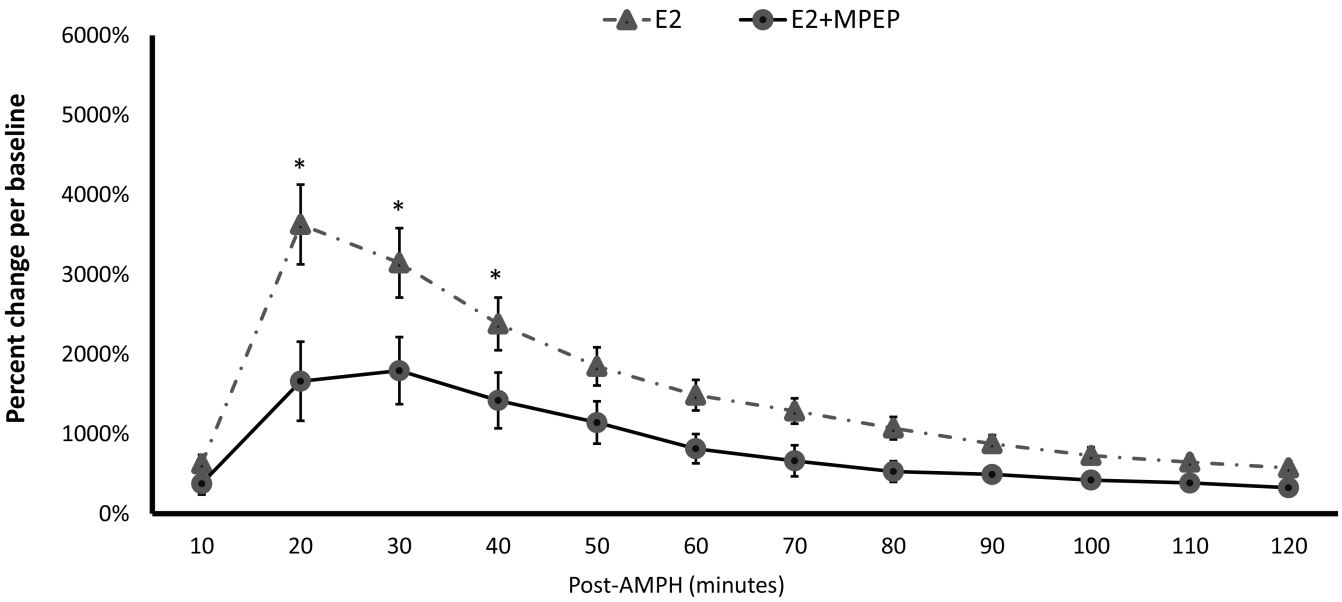
Exp 3	Treatment 1	Treatment 2	Group Size
E2	1 ng/mL E2 in 0.02% EtOH in Ringer's, inf.	1 mL/kg Saline, i.p.	n=9
E2+MPEP	1 ng/mL E2 in 0.02% EtOH in Ringer's, inf.	10 mg/kg MPEP in Saline @ 1 mL/kg, i.p.	n=9

Exp 4	Treatment 1	Treatment 2	Group Size
Control I *	1 mL/kg Saline, i.p.	0.1 mL Peanut Oil, s.c.	n=4
Control II *	0.1% EtOH in Ringer's, inf.	0.1 mL Peanut Oil, s.c.	n=4
MPEP	10 mg/kg MPEP in Saline @ 1 mL/kg, i.p.	0.1 mL Peanut Oil, s.c.	n=6
ICI	2.32 μg/mL ICI in 0.1% EtOH in Ringer's, inf.	0.1 mL Peanut Oil, s.c.	n=7

* Data combined







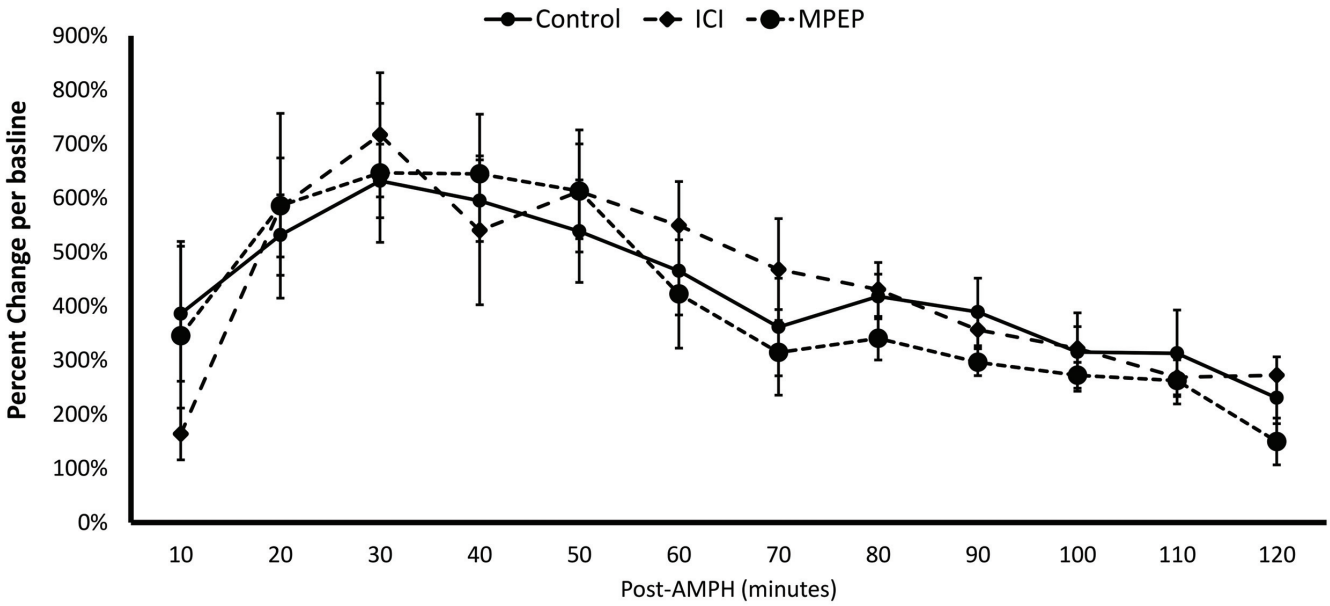


Table 1

ANOVA tests comparing rats treated with EB, E2 and Vehicle in Experiment 1				
Time post-AMPH	10min	20min	30min	40min
F value	F(2,25)=1.853	F(2,25)=4.433	F(2,25)=5.045	F(2,25)=3.939
p value	.178	**.023**	**.014**	**.033**
Planned contrast tests				
Time post-AMPH	10min	20min	30min	40min
P Value, Control VS EB	NA	**.037**	.083	0.233
P Value, Control VS E2	NA	**.013**	**.005**	**.010**
Mann-Whitney U tests comparing rats treated with EB and ICI+EB in Experiment 2				
Time post-AMPH	10min	20min	30min	40min
U	14.000	14.000	12.000	9.000
P value	**.034**	**.034**	**.021**	**.009**
Independent tests comparing rats treated with E2 and E2+MPEP in Experiment 3				
Time post-AMPH	10min	20min	30min	40min
t value	t(16)=1.410	t(16)=2.790	t(16)=2.232	t(16)=1.993
P value	.178	**.013**	**.040**	.064

Note: values in bold indicate significant differences.